TITLE:

***Mycoplasma genitalium* prevalence in Welsh sexual health patients: low antimicrobial resistance markers and no association of symptoms to bacterial load.**

AUTHORS:

Owen B. Spiller[1], Christopher L. Rees[1], Daniel J. Morris[1], Rebecca L. Davies[2], and Lucy C. Jones[1,2]

AFFLIATIONS:

1. Cardiff University, School of Medicine, Division of Infection and Immunity, Department of Medical Microbiology, University Hospital of Wales, Cardiff, CF14 4XN United Kingdom.
2. Department of Integrated Sexual Health, Dewi Sant Hospital, Cwm Taf Morgannwg University Health Trust Board, Pontypridd, CF37 1LB, United Kingdom.

Corresponding Author: Dr. Owen B. Spiller, Cardiff University, School of Medicine, Division of Infection and Immunity, Department of Medical Microbiology, University Hospital of Wales, Cardiff, CF14 4XN United Kingdom

Highlights:

1. First report of MG prevalence and antimicrobial resistance in Wales.
2. Second reported use of the SpeeDx prototype M. genitalium *parC* mutation assay.
3. M. genitalium bacterial load was not related to symptomatic patient presentation.
4. M. genitalium bacterial load between urine and swab samples are equivalent.
5. Gender and age bias identified in M. genitalium prevalence.

ABSTRACT:

Objectives: *Mycoplasma genitalium* (MG) is a common cause of sexually transmitted infection, however no prevalence data is available for Wales. MG was detected by qPCR (quantitative) as well as two separate SpeeDx commercial assays, and related to clinical symptoms, age, gender and sample type.

Methods: Cervical swabs, urethral swabs and/or urine were collected from 1000 patients at walk-in sexual health clinics at 3 Welsh health centres from October 2017-October 2018. Extracted DNA was investigated to determine concordance between an in-house quantitative PCR, SpeeDx ***Resistance****Plus*® MG and the SpeeDx MG+parC (beta 2) assays; mutations in *parC* were substantiated by Sanger sequencing.

Results: MG was detected in 17/600 female patients (2.7%) and 13/400 (3.5%) male patients, with a 100% concordance between in-house qPCR and both SpeeDx assays. Macrolide resistance was low (relative to other studies), but more common in males (4/13; 30.8%) than females (2/17; 11.8%) and the only fluoroquinolone resistant sample (3.4% overall) was also macrolide resistant and detected from an MSM. Vaginosis was clinically apparent in 12/17 MG-positive females (2 with additional cervicitis, 1 with additional pelvic inflammatory disease), while 7 MG-positive males were asymptomatic. MG bacterial load did not correlate to clinical symptoms and females (4,559 ± 1,646/ml) had significantly lower MG load than males (84,714 ± 41,813/ml; p=0.0429).

Conclusions: MG prevalence and antibiotic resistance in Welsh sexual health clinics is low. MG bacterial load did not correlate to clinical presentation, men have higher MG load/ml in urine than women, genders have different age bias for MG prevalence and urine and swabs are equivalent for detecting MG.

1. INTRODUCTION.

Mycoplasma genitalium (MG) is a 600-700 nm flask-shaped motile bacterium with a genome of approximately 580 kb that is transmitted by sexual contact [(1)]. It is generally accepted that MG infection causes urethritis in men [(1,2)] and is associated with increased risk of cervicitis, pelvic inflammatory disease, preterm birth, and infertility in women [(3)]. While the MG is gaining acceptance as a causative agent for these sexual health maladies, some authors postulate that only a minority of men infected with MG develop urethritis, while many remain asymptomatic [(2)]. The 2015 UK guidelines on non-gonococcal urethritis recommend not testing asymptomatic men [(4)].

Only two main population studies have provided general prevalence data for MG. In the US, a school-based random sampling of 18-27 year olds found 1.1% in 1218 men and 0.8% in 1714 women. While the Natsal-3 study examined a subset of 4507 urine samples from 15,162 randomly selected members of the UK public to find MG prevalence of 1.2% in men and 1.3% in women between the ages of 16-44 [(5)]. However, these studies were not designed to determine the ratio of symptomatic to asymptomatic individuals infected with MG. Bias in MG prevalence, investigating high versus low risk sexual activity groups was highlighted in a French study finding 4% MG in samples from presumptive high-risk sexual behaviour patients (STI, abortion, family planning and penitentiary centers) compared to 1.7% MG prevalence in gynaecological, obstetric and reproduction patients [(6)]. Studies examining MG prevalence in sexual health based environments have found higher rates: 5.3% (male) and 7.2% (female) in Western Canada (N=2254) [(7)], 17.2% (male) and 16.1% (female) in the US (N=964) [(8)], 10.3% (male) and 3.8% (female) in Denmark (N=7361) [(9)]. A study examining MG samples submitted at Swedish youth *Chlamydia trachomatis* (CT) and gonococcal (GC) screening clinics reported 9.8% MG prevalence, however, as only samples from 15–25 year olds [(10)] were investigated; this may skew the prevalence as 15–24 year olds are also known to have the highest CT prevalence. An aspect of MG prevalence that requires further clarification.

Treatment failure of MG with Azithromycin and moxifloxacin is a well-established trend: A poignant study in 2015 identified hallmark mutations in the 23S rRNA and topoisomerase/gyrase genes in isolates from patients with treatment failure [(11)]. Current studies are almost completely dependent on molecular methods to determine "resistant" MG infections (as defined by the presence of these mutations) as MG is extremely fastidious [(1,12)]. Key studies have combined *in vitro* MIC values to MG genomic sequences to validate the underlying mechanisms for the more frequently reported mutations in 23S rRNA, *gyrA* and *parC* genes [(13,14)]. Resistance-mediating mutations vary geographically as wells due to patient sexual demographics/risk behaviour. Macrolide resistance-mediating mutation (MRM) and fluoroquinolone resistance-mediating mutation (FRM) prevalence in 358 urethral MG isolates from symptomatic men attending Chinese sexual health clinics were found in 88.9% and 89.5% of sequenced isolates, respectively [(15)]. At the other extreme no MRM and only 0.4% established FRM prevalence rates were reported for 266 MG isolates from HIV and STI patients in South Africa [(16)].

Here we report the first MG prevalence study in Wales, investigating 1000 participants attending 3 walk-in sexual health clinics from October 2017 to September 2018. Evidence of MG infection was correlated between an in-house qPCR assay and the commercial SpeeDx ***Resistance***Plus® MG assay to determine the MG MRM prevalence. We also present the results from the prototype SpeeDx MG+parC (beta 2) assay relative to Sanger sequencing of the *parC* gene for all isolates. We also correlate the MG bacterial load to clinical symptoms in the participants, MG bacterial loads between genders and sample type, as well as examine differing age biases for MG prevalence between men and women.

2. MATERIALS AND METHODS.

2.1 Participant samples. First-void Urine and/or swabs (urethral, endocervical or high vaginal swabs) were collected from informed and consented participants from October 2017 to October 2018 attending Welsh sexual health walk-in clinics held at Dewi Sant hospital (Pontypridd), Keir Hardie Health Park (Merthyr Tydfil), or Ysbyty Cwm Cynon (Mountain Ash). Samples were obtained following informed consent, from a cross-section of all patients attending walk-in sexual health clinics, under

ethical approval (IRAS 230693) and results of analysis for MG were linked to patient notes under subsequent ethical approval (IRAS 253889). Six hundred female participants were recruited (symptomatic female patients included 31 with pelvic inflammatory disease, 16 with cervicitis and 176 with vaginitis) and four hundred male participants were recruited including 84 male patients with urethritis (69 of which were negative for both Chlamydia or gonococcus), 9 with balanitis (6 exclusive of urethritis) and 22 with epididymitis (21 exclusive of urethritis or balanitis) summarised in Table 1. All patients presenting with symptoms were additionally investigated for the presence of *Chlamydia trachomatis* (CT) or *Neisseria gonorrhoea* (GC) by standard NHS diagnostic procedure by submitting a urine or swab (as appropriate) sample using Roche diagnostics samples for cobas® CT/NG assay testing utilising a cobas® 4800 (Roche Molecular Systems, Pleasanton, CA).

Table 1. Bacterial infections and clinical diagnoses of 1000 Sexual health walk-in participants.

FEMALES	Total	Vaginitis	PID	Cervicitis	None of these
Total in 600	600	203	31	16	395
CT	30	24	1	1	6
GC	8	6	0	1	2
CT+GC	2	2	0	0	0
Syphilis	1	0	0	0	1
MG	17	12	1	2	5
MG+CT	2	2	0	0	0
MG+CT+GC	1	1	0	0	0
MALES	Total	Urethritis	Balanitis	Epididymitis	None of these
Total in 400	400	84	9	22	285
CT	20	0	0	1	19
GC	6	4	0	0	2
Syphilis	4	0	0	0	4
MG	14	7	0	0	7
MG + CT or MG	0	0	0	0	0

Legend: CT = *Chlamydia trachomatis*, GC= *Neisseria gonorrhoea* (gonococcus), MG= *M. genitalium*.

2.2 Clinical evaluation of participants: Patients with a female reproductive system were clinically assessed by a sexual health specialist and symptoms, signs on examination, and specific investigations were used to make a diagnosis in accordance with guidelines set out by the British Association of Sexual Health and HIV (BASHH) and Royal College of Obstetrics and Gynaecology, as listed in Supplementary table 1; e.g. bacterial vaginosis, non-specific vaginitis, cervicitis or pelvic

inflammatory disease (PID). Similarly, patients with a male reproductive system were diagnosed with urethritis, balanitis or epididymitis using BASHH guidelines as listed in Supplementary table 1. Participants recruited in walk-in clinic without symptoms relevant to MG infection included those with complex contraceptive needs, genital wart treatments, herpes infection and genital dermatoses. All findings, diagnoses and treatments were recorded in the participant's notes along with clinical history and demographic parameters (e.g. age, gender identity, the number of sexual contacts, whether patients engaged in oral, vaginal or anal sex etc.) and notes were retrospectively linked to research laboratory findings at the conclusion of patient recruitment.

2.3 Sample processing. Urine samples were processed as follows: 2 ml of urine were centrifuged at 15,000*xg* for 20 min, supernatant removed and pellet frozen at -86^{o}C until extraction of DNA using the Qiagen QiaCube automated system. Swab samples were processed as follows: Copan Rayon swab samples were either taken by the clinician (endocervical) or self-taken by the participant (high vaginal), material was re-suspended in 10 ml sterile saline (CPM S.A.S., Rome, Italy) within 20 min and 2 ml centrifuged and processed for DNA extraction as above. DNA extracts were stored at -86^{o}C until batch analysed by quantitative PCR.

2.4 Molecular analysis. Analysis of the previously published MgPa380 probe [(17)] determined that its calculated Tm=51.5^{o}C and the accompanying MgPa355F primer has a calculated Tm=59.1^{o}C (but also is predicted to have a hairpin with a Tm=48.9^{o}C) and is found to have a one SNP mismatch compared to the whole genome sequence for strain M6282 (Genbank accession: CP003771). Therefore new primers and probes conserved between all published MG sequences with Tm≥60^{o}C were designed: Primers mgpB_BnB_FP GAAGGTATGATAACAACGGTAGAGCTT and mgpB_BnB_RP CCATTTAAACCCCTTTGCACCGT with Taqman hydrolysis probe mgpB_BnB_HP [CY5]-ACTTAGCAAAAATGGAAAACCCCTC-[BHQ650]. Five microliters of extract were added to 15µL SsoAdvanced Universal Probes Supermix (Bio-Rad Laboratories) and 1 cycle of 5 min at 95^{o}C, then 45 cycles of 15 seconds at 95^{o}C and 15 seconds at 60^{o}C were analysed by integral CFX Maestro™ Software

in the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories). Genome copies were calculated against a 6-log dilution curve (10^6-10^1 copy) of the G37 strain (accession NC_000908.2) P1 gene (nucleotides 350-481), synthesised by GenScript Biotech (Netherlands). Five microliters were additionally analysed using the SpeeDx ResistancePlus MG assay and MG positive samples were additionally analysed by the SpeeDx MG+parC (beta 2) assay for fluoroquinolone *parC* mutations A247C (S83R), G248T (S83I), G248A (S83N) or G259T (D87N), G259A (D87N) or G259C (D87H). Results for SpeeDx assays were analysed according to the manufacturer's instructions and *parC* sequence was confirmed by Sanger sequencing (Source Biosciences, Cambridge UK) amplicons generated using parC-MG_RP533 ATCCAGCAGCTATCCCACTC and parC-G37_FP with GCTTAAAACCCACCACTCCC or parC-M6282-FP GCTTAAAACCCACCACTTCCT.

2.5 Statistical analysis. All statistical analyses were performed using GraphPad Prism version 7 (GraphPad Software). Descriptive statistics (i.e. bacterial load per gender, etc.) were presented as average ± standard deviation and prevalence ranges included 95% confidence interval ($CI_{95\%}$). MG bacterial loads were $\log_{10}$ transformed to fit a normal distribution and correlations were calculated using Pearson correlation assuming a Gaussian distribution and results were presented as R^2 values, which were highly significant ($P<0.0001$). Significance was only attributed to comparisons where P value <0.05.

3. RESULTS.

3.1 Prevalence of *M. genitalium* in Wales. Thirty individual participants from the recruited 1000 sexual health clinic walk-in patients were found to be positive for *M. genitalium* (MG). All MG-positive samples were detected by the SpeeDx ResistancePlus MG assay, the SpeeDx MG+parC (beta 2) assay and the in-house BnB_MgPa Taqman hydrolysis probe qPCR assay. MG-positive samples were found in 17/600 female patients (2.7%; $CI_{95\%}$=1.5-4.0%) and 13/400 (3.5%; $CI_{95\%}$=1.7-5.2%) male patients. Eleven of the male participants were heterosexual and 2 were MSM (men who have sex with men). Only one male participant was detected on two separate occasions in samples taken 1 month apart (i.e. 14 separate male samples positive for MG). Quantitative PCR measurement of MG

Bacterial load measured genomic copy number to be 4,559 ± 1,646 per ml sample provided for all female participants and 84,714 ± 41,813 per ml for male participants (**Figure 1A**; p=0.0429; t-test). Of the 17 MG-positive female participants, 12 had clinical evidence of vaginitis, 2 had cervicitis and 1 was diagnosed with pelvic inflammatory disease, while 5 were asymptomatic (29.4%). Two of the MG-infected female participants with vaginitis were found to be co-infected with CT and an additional participant with vaginitis was co-infected with both CT and GC (Table 1); however, CT and GC were absent from the 3 cases of PID and cervicitis. Of the 14 MG-positive male samples, none were co-infected with CT or GC and 7 had clinical evidence of non-specific (or non-gonococcal) urethritis (50%), while 7 were asymptomatic (as the participant with two positive samples was symptomatic on his first visit, but asymptomatic on his follow-up visit).

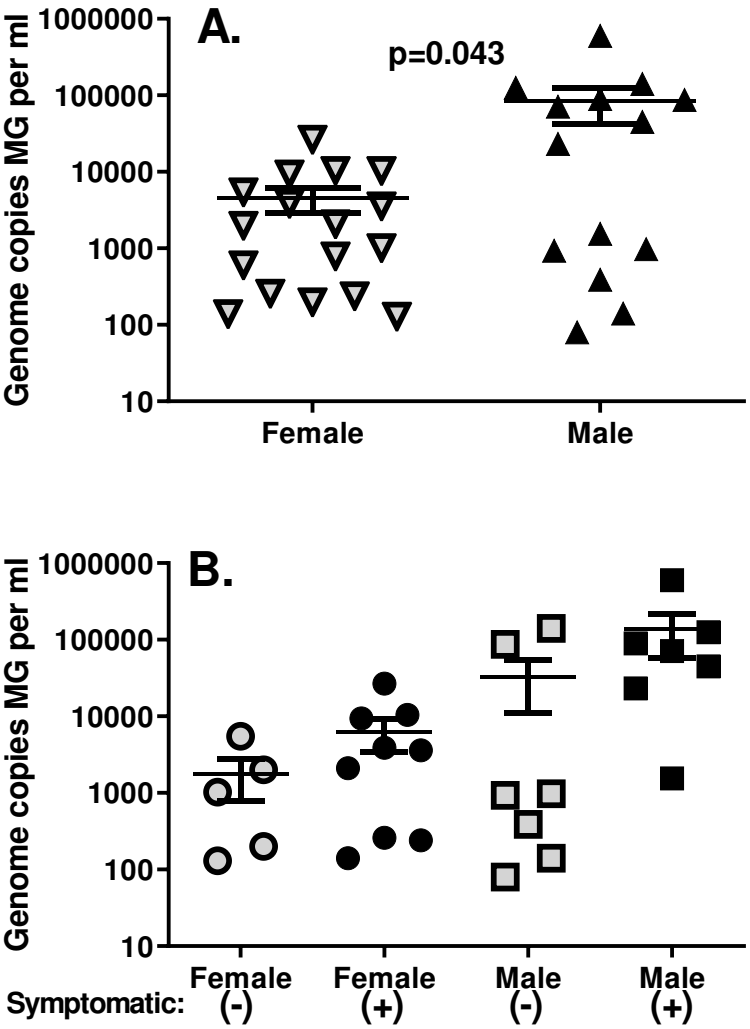


Figure 1. *M. genitalium* bacterial load, as determined by Taqman hydrolysis probe qPCR measurement against a 6-point standard curve. **A.** Difference in MG copies/ml between male and females (p=0.043). **B.** Results segregated between asymptomatic female patients compared to those with vaginitis, cervicitis and pelvic inflammatory disease diagnoses and asymptomatic male patients compared to those with urethritis diagnoses.

3.2 Symptomatic presentation is not related MG bacterial load. No relationship could be found between MG load and symptomatic presentation (co-infected participants excluded) (**Figure 1B**): asymptomatic females had 1,770 ± 992 (range 130-5,500) MG Geq/ml compared to 5,721 ± 2,240 (range 140-26,750) MG Geq/ml (p=0.288) in participants with vaginosis and asymptomatic males had 32,840 ± 21,649 (range 80-140,000) MG Geq/ml compared to 136,587 ± 78,807 (range 1,550-600,000) MG Geq/ml (p=0.228) in those with urethritis. Of the 17 MG-positive female patients, 6 samples were submitted as swabs only, 6 provided a urine sample only and 5 provided both sample types that were processed individually. One of these was MG-positive for swab sample only (236 Geq/ml) and another was MG-positive for urine sample only (3880 Geq/ml), while the remaining were MG-positive for both sample types (**Supplementary figure 1**). No consistent pattern for higher MG levels between sample type were apparent in these three dual MG-positive female samples. While the average MG copy number appeared higher for all female urine samples (5,525 ± 2,547 Geq/ml) relative to all female swab samples (2,636 ± 1,240 Geq/ml), this comparison failed to achieve statistical significance for this small sample size.

3.3 Gender and age bias in MG prevalence. The average age was 28.8 years ($CI_{95\%}$=28.0-29.6) for female participants and 30.9 years ($CI_{95\%}$=29.8-32.0) of age for males (range 16-68 for both genders). More participants were present for 16-24 years (36.0% females; 41.5% males) and 25-34 years (36.5% females; 34.8% males) compared to older age groups of 35-44 (14.4% females; 15.3% males) and over 45 (13.1% females; 8.3% males). While MG infection did not differ by gender, a general trend in age demographics was observed: a steady decline in percent MG infection was observed from the 16-24 age group in female participants, while MG infection rates increased to peak in the

35-44 age group in men, before dropping sharply off (**Figure 2**). While the numbers of infected patients are small, it is notable that this was the exact trend observed in a French study of 2652 samples collected from 2014-2015 [(6)] (**Figure 2**).

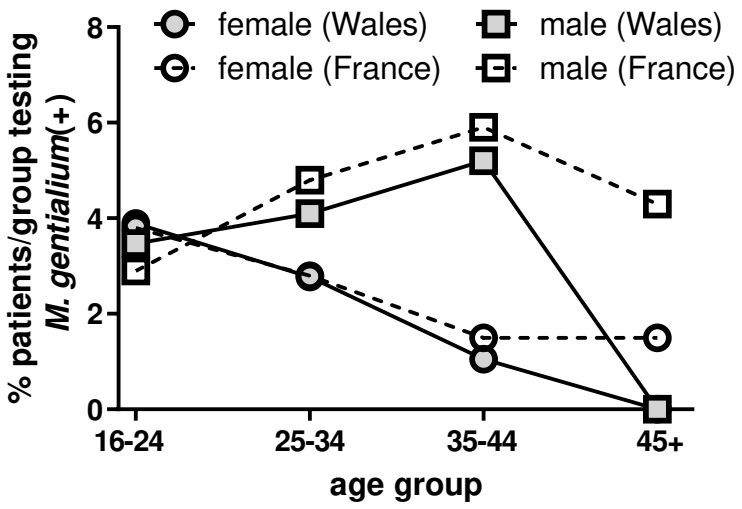


Figure 2. *M. genitalium* prevalence as a percentage of patients investigated stratified by age group. Male patients identified as grey squares and female patients identified as grey circles. All patients determined as positive by SpeeDx ResistancePlus MG, SpeeDx MG+parC (beta 2) and our in house qPCR recognising the major surface antigen MgPa. Data for French patients (2014-2015) reported previously [(6)] shown in open symbols with dotted lines for comparison.

3.4 Concordance between bacterial load and commercial assay Cq value. There was a complete concordance for MG detection between in-house qPCR and the results for the SpeeDx ResistancePlus MG and SpeeDx MG+parC (beta) assays (including the MG-negative female swab and urine sample from matched samples above). The Cq values for MgPa detection in each assay nearly identical between the ResistancePlus MG and SpeeDx MG+*parC* (beta 2) assays; furthermore, these Cq values correlated very closely to a $\log_{10}$ transformation of the Geq values determined by our in-house assay for the same samples (**Figure 3**; Pearson R^2=0.933). This indicates that the cycle number at which the SpeeDx probe for MgPa crosses the threshold can give a rough indication of how high the MG load is: 10 MG Geq per well was consistently about 27 cycles, 100 Geq per well about 23.5

cycles, 1000 Geq per well about 21 cycles, and any samples ≤17 cycles were consistently ≥10,000 Geq per well.

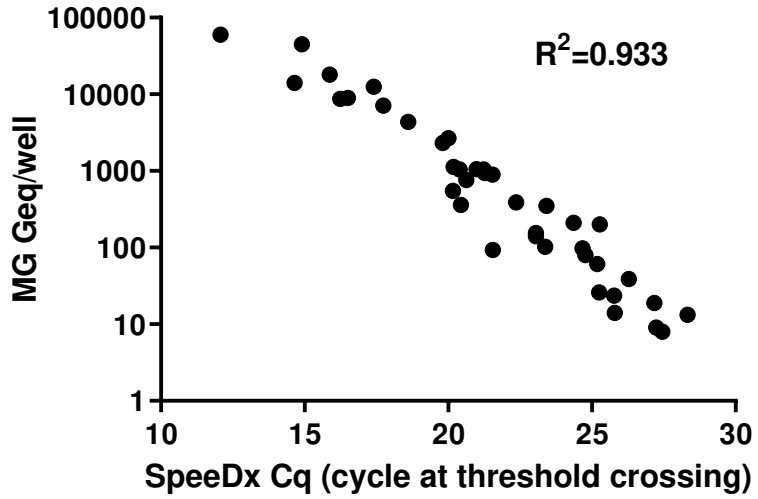


Figure 3. Correlation between SpeeDx MgPa probe Cq (calculated cycle number crossing positive threshold) and genomic copy number (Geq) per well as determined by our in-house Taqman hydrolysis probe (determined against a 6-point standard curve).

3.5 Confirmation of commercial ParC mutation results by Sanger sequencing. The SpeeDx ResistancePlus MG assay is currently a commonly used and accepted *in vitro* diagnostic utilised for identifying macrolide resistance-mediating mutations (MRM) [(18)]. In our cohort 4/13 male patients had MRM (30.8%) and 2/17 female patients had MRM (11.8%) giving an overall MRM prevalence of 20%. We also tested the prototype SpeeDx MG+parC (beta 2) molecular test for determining the presence of the most common ParC mutations associated with fluoroquinolone resistance (S83I, S83R, S83N, D87N, D87Y, and D87H). Screening the 30 MG-positive samples in our 1000 patient cohort we only identified one patient (3.3%) with an S83I/S83R and Sanger sequencing of this isolate confirmed the presence of an A248T (S83I) mutation, in an isolate that concurrently contained an MRM mutation (16.6% of 6 MRM-MG positive samples).

3.6 Anecdotal observations from repeat samples in patient subset. Post-study analysis (MG-testing was blinded and clinical MG testing was not available in the clinics at the time of study) allowed us to

follow five patients (Supplementary figure 2): A symptomatic male patient with MG load of 23,180 Geq/ml sample, was treated with doxycycline for 7 days and was asymptomatic on follow-up 28 days later despite still having an MG load of 87,360 Geq/ml (patient had no other detectable organisms in their samples, MG was macrolide sensitive). A second symptomatic male patient with an MG load of 71,220 Geq/ml sample, was treated with 2g azithromycin over 4 days and was asymptomatic with no detectable MG 2 weeks later (MG was macrolide sensitive). A symptomatic female patient with an MG load of 10,410 Geq/ml (urine) and 670 Geq/ml (swab) was treated with doxycycline and metronidazole for 7 days and returned asymptomatic with no detectable MG 3 weeks later (macrolide resistant MG). A final participant being treated for herpesvirus that did not have urethritis, had an MG load of 390 Geq/ml and self-resolved without use of antibiotics 28 days later. A single participant (one of two MG-positive MSM participants) in this study was asymptomatic with an MG load of 980 Geq/ml returned and was still asymptomatic 9 months later (participating in a subsequent study) with an MG load of 56 Geq/ml; worryingly both samples contained combined macrolide and fluoroquinolone resistance markers.

4. DISCUSSION.

To date, several small-scale prevalence studies have been reported and MG prevalence estimates vary widely. An unbiased population sampling in the UK (Natsal-3) from 2010-2012 determined the MG rates to be 1.2% in men and 1.3% in women [(5)], while MG prevalence in sexually active young women (17-27 years old) in England between 2004-2008 was found to be 3.3% [(19)]. A recent French study has shown that significant difference is to be expected between sampling of low-risk (1.7% MG) and high-risk (4.0% MG) sexual activity biased populations [(6)]. Here we present the first MG prevalence study for 1000 participants attending Welsh walk-in sexual health clinics: MG was detected in 2.8% of female participants (4.2% of the 17-27 age group) and 3.3% of males. Differences observed in Welsh MG prevalence trends between the genders for various age groups also closely mirrored observations in French

patients [(6)] (Figure 3). This same gender-biased MG trend can also be observed (albeit with lower prevalence) in the unbiased Natsal-3 UK data [(5)].

The rapid global emergence of antimicrobial resistance (with limited therapeutic options) has propelled development of MG detection with antimicrobial resistance (AMR) determination to a high international priority. The SpeeDx ResistancePlus MG assay was found to have a specificity of 100%, sensitivity of 94.9% and an accuracy for determination of MRM of 92.4% for MG-positive samples from England [(18)]. We found specificity and sensitivity of 100% relative to our in-house qPCR, with a close correlation between the Cq values for the MgPa gene in SpeeDx assays to our calculated MG bacterial load (R^2= 0.933). We also report the second use of SpeeDx MG+parC (beta 2) assay in the literature [(20)] (this is the first in the UK), and we found a complete concordance with *parC* Sanger sequencing of all isolates.

Similar to recent reports from Australia and Canada [(7,21)], we found MG-MRM rates higher in males (30.8%) compared to females (11.8%). However, our overall MRM rate (20%) was much lower than the 82.4% MRM recently reported for England [(22)], as well as those reported recently for China (88.9% [(15)]), Queensland Australia (75.9% [(17)]), New Zealand (77.4% [(23)]), Alberta Canada (66.2% [(7)]), and Germany (52.6% [(24)]). Our 3.3% overall fluoroquinolone resistance mutation (FRM) rate was similar 4.9% to England (4.9% [(22)]). FRM rates also vary geographically: 0.4% in South Africa [(16)], 4.7% in France [(25)], 10.5% in Germany [(24)], 12.2% in Western Canada [(7)], 23.3% in New Zealand [(23)] and 89.5% in China [(26)]. MG AMR rates have previously been reported for MSM populations [(11,21,27)], and it is notable that our only dual resistant MG isolate was found in a sample from an MSM participant. However, we found the overall MG rates roughly the same between MSM (2/79; 2.5%) and heterosexual men (12/321; 3.4%), as expected based on other reports [(27)].

Opinion varies widely regarding asymptomatic carriage of MG, often obscured by the use of self-reported symptoms in some reports compared to classification by sexual health clinicians in others.

A recent review suggested that infection in men is usually asymptomatic and it is likely that most men resolve infection without developing disease [(2)] and contemporary study groups have highlighted the need for unlinked anonymous monitoring of MG in sexual health populations [(28)]. Retrospective linking of MG results to physician-determined clinical status of participants in our study identified that 50% MG-positive male participants had urethritis (no co-infections were observed), while 71% of female participants had vaginitis (64% with CT and GC co-infections excluded). This compares to 10% asymptomatic MG-infection in New Zealand [(23)], 70.9% asymptomatic MG-infections in France [(6)], 58-60% asymptomatic MG-infections in Western Canada [(7)] and 17% asymptomatic females versus 55% asymptomatic males enrolled in clinics across the USA [(8)]. However, proctitis does not appear to be caused by MG infection [(29)] despite MG rates being almost 3 times more frequent relative to urine samples for some populations. Most importantly, we found that there was no significant correlation between clinical presentation (urethritis or vaginitis compared to asymptomatic patients) and MG bacterial load in our study (Figure 1), which is in contrast to a previous report [(30)]. We did identify one asymptomatic male participant with very low MG bacterial load that resolved without any therapy as well as a second male participant that had very low MG bacterial loads in samples 9 months apart. MG bacterial load was significantly higher in men than in women (**Figure 1A**), but this was not due to sample type bias since swabs and urine samples were comparable in matched samples from women (**Supplementary Figure 1**). Gratrix *et al.* [(7)] also found a 98.1% concordance in MG detection between urine and swabs taken from the same patient and also found both swab and urine samples from women occasionally missed MG detection in matched samples. Therefore, unlike CT screening MG detection is not superior in swab samples for women.

In conclusion, our study has the same limitations common to all broad population screening studies where participants are a combination of symptomatic patients seeking treatment, asymptomatic individuals requiring complicated contraception, worried well, contact tracing, and those requiring health monitoring due to long-term therapy, with the bias that they represent a sexually active

cohort. We observed an MG prevalence concordant to other international studies, with the same gender biased trends in age groups as other studies. We found lower AMR rates in MG compared to those reported in England [(22)] and found that MG bacterial load is not correlated to symptomatic clinical presentation. We found one asymptomatic participant with low MRM/FRM MG levels detected 9-months apart; longer term follow-up of these patients is required to see if chronic MG carriage eventually manifests as symptomatic infection and the importance of asymptomatic individuals passing antimicrobial resistant strains to other sexual contacts.

REFERENCES

1. Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: From chrysalis to multicolored butterfly. Clin Microbiol Rev. 2011;24(3):498–514.
2. Horner PJ, Martin DH. *Mycoplasma genitalium* Infection in Men. J Infect Dis. 2017;216(Suppl 2):S396–405.
3. Lis R, Rowhani-Rahbar A, Manhart LE. Mycoplasma genitalium Infection and Female Reproductive Tract Disease: A Meta-analysis. Vol. 61, Clinical Infectious Diseases. 2015. p. 418–26.
4. Horner P, Blee K, O'Mahony C, Muir P, Evans C, Radcliffe K. 2015 UK National Guideline on the management of non-gonococcal urethritis. Int J STD AIDS. 2016;27(2):85–96.
5. Sonnenberg P, Ison CA, Clifton S, Field N, Tanton C, Soldan K, et al. Epidemiology of *Mycoplasma genitalium* in British men and women aged 16-44 years: Evidence from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). Int J Epidemiol. 2015;44(6):1982–94.
6. Pereyre S, Laurier Nadalié C, Bébéar C, Arfeuille C, Beby-Defaux A, Berçot B, et al. *Mycoplasma genitalium* and *Trichomonas vaginalis* in France: a point prevalence study in people screened for sexually transmitted diseases. Clin Microbiol Infect. 2017;23(2):122.e1-122.e7.
7. Gratrix J, Plitt S, Turnbull L, Smyczek P, Brandley J, Scarrott R, et al. Prevalence and antibiotic resistance of *Mycoplasma genitalium* among STI clinic attendees in Western Canada : a cross-sectional analysis. 2017;1–8.
8. Getman D, Jiang A, Donnell MO. Antibiotic Resistance Frequency in a Multicenter Clinical Study Cohort in the United States. J Clin Microbiol. 2016;54(9):2278–83.
9. Salado-Rasmussen K, Jensen JS. *Mycoplasma genitalium* testing pattern and macrolide resistance: A Danish nationwide retrospective survey. Clin Infect Dis. 2014;59(1):24–30.
10. Nolskog P. STI with *Mycoplasma genitalium* — more common than *Chlamydia trachomatis* in patients attending youth clinics in Sweden. 2019;81–6.
11. Bissessor M, Tabrizi SN, Twin J, Abdo H, Fairley CK, Chen MY, et al. Macrolide resistance and azithromycin failure in a *mycoplasma genitalium*-infected cohort and response of azithromycin failures to alternative antibiotic regimens. Clin Infect Dis. 2015;60(8):1228–36.
12. Fookes MC, Hadfield J, Harris S, Parmar S, Unemo M, Jensen JS, et al. Mycoplasma genitalium : whole genome sequence analysis , recombination and population structure. 2017;1–11.
13. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin Treatment Failure in *Mycoplasma genitalium* – Positive Patients with Nongonococcal Urethritis Is Associated with Induced Macrolide Resistance. 2008;47.
14. Fernandes P, Unemo M. In Vitro Activity of the New Fluoroketolide Solithromycin (CEM-101) against Macrolide-Resistant and -Susceptible *Mycoplasma genitalium*. 2014;58(6):3151–6.
15. Li Y, Su X, Le W, Li S, Yang Z, Chaisson C, et al. *Mycoplasma genitalium* in symptomatic male urethritis : macrolide use is associated with increased resistance.
16. Muller EE, Mahlangu MP, Lewis DA, Kularatne RS. Macrolide and fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium* in Johannesburg, South Africa, 2007-2014.

BMC Infect Dis. 2019;19(1):1–8.

17. Jensen JS, Björnelius E, Dohn B, Lidbrink P. Use of TaqMan 5' nuclease real-time PCR for quantitative detection of *Mycoplasma genitalium* DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. J Clin Microbiol. 2004;42(2):683–92.

18. Pitt R, Cole MJ, Fifer H, Woodford N. Evaluation of the Mycoplasma genitalium Resistance Plus kit for the detection of *M. genitalium* and mutations associated with macrolide resistance. Sex Transm Infect. 2018;94(8):565–7.

19. Oakeshott P, Aghaizu A, Hay P, Reid F, Kerry S, Atherton H, et al. Is *Mycoplasma genitalium* in Women the "New Chlamydia?" A Community-Based Prospective Cohort Study . Clin Infect Dis. 2010;51(10):1160–6.

20. Fernandez-Huerta M, Bodiyabadu K, Esperalba J, Bradshaw CS, Serra-Pladevall J, Garland SM, et al. Multicenter clinical evaluation of a novel multiplex real-time PCR (qPCR) assay for detection of fluoroquinolone resistance in *Mycoplasma genitalium*. J Clin Microbiol. 2019 Aug;

21. Sweeney EL, Trembizki E, Bletchly C, Bradshaw CS, Menon A, Francis F, et al. Levels of *Mycoplasma genitalium* Antimicrobial Resistance Differ by Both Region and Gender in the State of Queensland, Australia: Implications for Treatment Guidelines . J Clin Microbiol. 2019;57(3):1–10.

22. Pitt R, Fifer H, Woodford N, Alexander S. Detection of markers predictive of macrolide and fluoroquinolone resistance in *Mycoplasma genitalium* from patients attending sexual health services in England. 2018;9–13.

23. Anderson T, Coughlan E, Werno A. *Mycoplasma genitalium* Macrolide and Fluoroquinolone Resistance Detection and Clinical Implications in a Selected Cohort in New Zealand. J Clin Microbiol. 2017;55(11):3242–8.

24. Dumke R, Thürmer A, Jacobs E. Emergence of *Mycoplasma genitalium* strains showing mutations associated with macrolide and fl uoroquinolone resistance in the region. Diagn Microbiol Infect Dis [Internet]. 2016;86(2):221–3. Available from: http://dx.doi.org/10.1016/j.diagmicrobio.2016.07.005

25. Le Roy C, Hénin N, Pereyre S, Bébéar C. Fluoroquinolone-Resistant *Mycoplasma genitalium*, Southwestern France . Emerg Infect Dis. 2016;22(9):1677–9.

26. Li Y, Su X, Le W, Li S, Yang Z, Chaisson C, et al. *Mycoplasma genitalium* in symptomatic male urethritis : macrolide use is associated with increased resistance. Clinical Infectious Diseases 2019. In press. https://doi.org/10.1093/cid/ciz294

27. McIver R, Jalocon D, McNulty A, Jeoffreys NJ, Chen SC-A, Power M, et al. Men who have sex with men with *Mycoplasma genitalium*-positive nongonococcal urethritis are more likely to have macrolide resistant strains than men with only female partners: a prospective study. Sex Transm Dis. 2019 Apr;

28. Bradshaw CS, Horner PJ, Jensen JS, White PJ. Syndromic management of STIs and the threat of untreatable *Mycoplasma genitalium*. Lancet Infect Dis. 2018;18(3):251–2.

29. Read TRH, Murray GL, Danielewski JA, Fairley CK, Doyle M, Worthington K, et al. Symptoms, Sites, and Significance of *Mycoplasma genitalium* in Men Who Have Sex with Men. Emerg Infect Dis. 2019;25(4):719–27.

30. Yoshida T, Deguchi T, Ito M, Maeda S, Tamaki M, Ishiko H. Quantitative Detection of *Mycoplasma genitalium* from First-Pass Urine of Men with Urethritis and Asymptomatic Men by Real-Time PCR. 2002;40(4):1451–5.

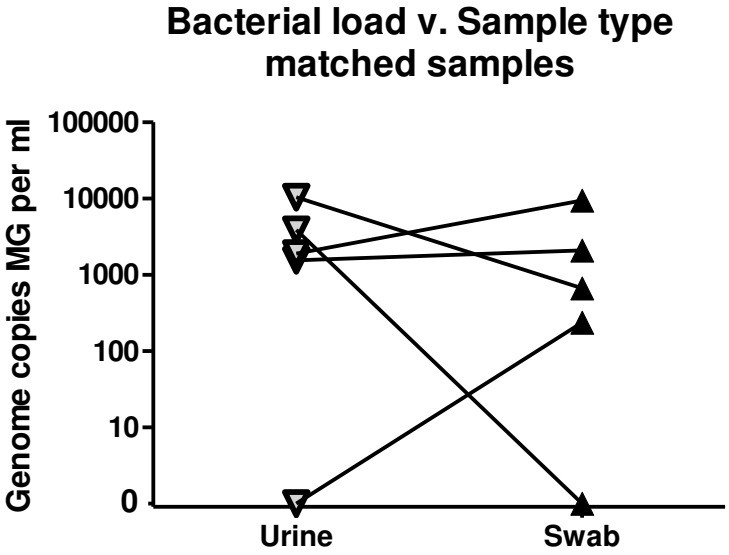
Acknowledgements.

We would like to thank Professor John Geen (Deputy Director of the Cwm Taf Morgannwg University Health Board Trust Research and Development Department) for support and arranging sponsorship for this clinical study, as well as the R&D research officers and research nurses, especially Rhian Beynon, Lisa Roche, Meryl Rees, Kelly Thomas and Heidi Clarke for consenting patients and administrative support. The NIHR Clinical Research Network for adopting our study to the UK clinical portfolio. Clinical nurses Suzanne Williams and Yvonne Burton. This study was funded by the Knowledge Economy Skills Scholarship 2 (KESS2) programme of funding for Daniel J Morris under the supervision of Owen B Spiller and Lucy C Jones.

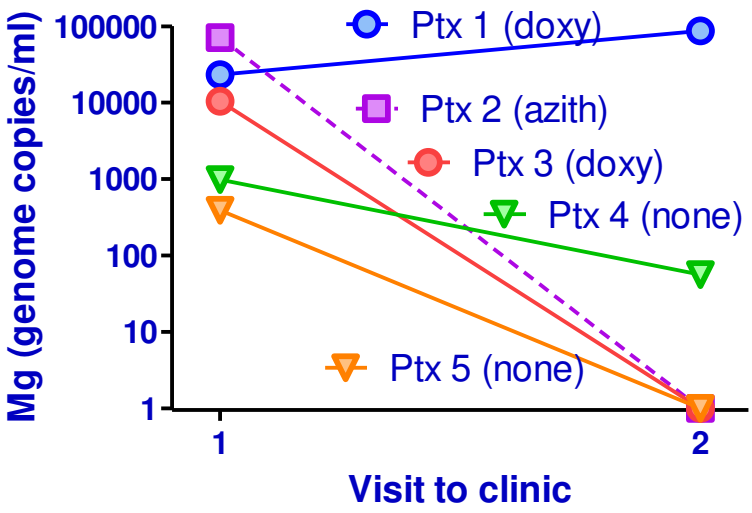
Supplementary Table 1. DIAGNOSTIC CRITERIA USED FOR SYMPTOMATIC PATIENTS IN ACCORDANCE WITH BASHH GUIDELINES

DIAGNOSIS	SYMPTOMS	SIGNS	INVESTIGATIONS
URETHRITIS	Urethral discharge, dysuria, penile irritation, urethral discomfort.	Muco-purulent urethral discharge, balano-posthitis	5 or more PMNL's per high power microscopic field on urethra smear. Threads in 1st pass urine; +/- >1+ leucocyte esterase dipstick on FPU.*
BACTERIAL VAGINOSIS	Abnormal vaginal discharge. Absence of pruritus, soreness or irritation.	Thin homogenous discharge pH>4.5 with no macroscopic evidence of inflammation. No vaginal erythema or oedema.	HVS microscopy and culture. No organisms detected on direct plating to fungal media. *Chlamydia* and *Gonorrhoea* NAAT negative..
NON-SPECIFIC VAGINITIS	Vulval pruritus, vulval soreness, abnormal vaginal discharge, vaginal discharge, superficial dyspareunia, external dysuria. No history of vulval dermatoses or allergy.	Vaginal discharge, vulval erythema +/- oedema, erythema of vaginal walls. Absence of signs of dermatological conditions or allergy.	HVS for microscopy and culture. No organisms on *Candida* testing (direct plating to fungal media), *Gardnerella* and TV. *Chlamydia* and *Gonorrhoea* NAAT, Syphilis serology.
CERVICITIS	Abnormal discharge, post-coital bleeding +/- intermenstrual bleeding, dyspareunia. Absence of CIN or cervical neoplasia.	Purulent endocervical exudate, contact bleeding of ectocervix in absence of ectropion, strawberry cervix.	Microscopy and culture of endocervical swab. 30 or more PMNL per high power field. No organisms detected on *Candida* testing (direct plating to fungal media), *Gardnerella* and TV. *Chlamydia* and *Gonorrhoea* NAAT.
PID	Abnormal vaginal discharge, deep dyspareunia, lower abdominal/pelvic pain, post-coital bleeding +/- abnormal vaginal bleeding. Absence of co-existing gynaecological conditions	Adnexal tenderness, cervical motion tenderness, pain on bimanual examination of uterus.	5 or more pus cells on endocervical microscopy. No organisms detected on Candida testing (direct plating to fungal media), *Gardnerella*, TV, *Chlamydia* and *Gonorrhoea* NAAT, Syphilis serology.
EPIDIDYMITIS	Scrotal pain (unilateral), swelling +/- urethral discharge.	Tenderness and swelling of epididymis, +/- urethral discharge, absence of spermatocoele /herniae /torsion /varicocoele.	Urethral swab for microscopy and culture. Urine for CT/NG NAAT. Urine dipstick negative (eliminated UTI).

*Not all male participants had leucocyte esterase dipstick on FPU.



Supplementary Figure 1. *M. genitalium* bacterial load as determined by in house qPCR for matched urine and swab samples from 5 female patients. MG was only detected in one patient in the swab sample and only detected in the urine sample of another patient. Of the remaining 3 patients MG bacterial loads were similar for both samples.



Supplementary Figure 2. *M. genitalium* bacterial load as determined by in house qPCR participants (Ptx) with between visits to clinic from participants (antibiotics received between visits are given in parentheses for Ptx 1, 2 and 3. MG was detected in two participants that were asymptomatic, one of which self-resolved (Ptx 5), as results were not made available to the clinicians no treatment was required for these two individuals. Time between samples: Ptx1 = 4 weeks, Ptx2 =2 weeks, Ptx3= 3 weeks, and Ptx5 = 4 weeks. It is notable that the time between visits for Ptx 4 was 9 months and this isolate was the only dual macrolide and fluoroquinolone resistant isolate in the study.